Analysis of Ascorbic Acid by Electrochemical Detection

*L. Z. Pei, T. Wei, N. Lin, Z. Y. Cai

Key Lab of Materials Science and Processing of Anhui Province,
School of Materials Science and Engineering,
Anhui University of Technology, Ma'anshan, Anhui 243002, People's Republic of China
*e-mail: lzpei1977@163.com ; lzpei@ahut.edu.cn

A glassy carbon electrode (GCE) modified by copper-doped titanium dioxide nanoneedles has been fabricated and used for the electrochemical detection of ascorbic acid (AA) in KCl solution. Two pairs of peak currents on quasi-reversible electrochemical cyclic voltammogram peaks (cvps) are located at +0.16 V, -0.03 V (cvp 1 and cvp 2) and +0.01 V, -0.44 V (cvp 1′ and cvp 2′), respectively. The relationship between the peak current and AA concentration is linear in the concentration range from 0.0005 to 2 mM. There is also a linear relationship between the peak current and the scan rate. The detection limit is 0.37 μM and 0.25 μM for cvp 1 and cvp 2, respectively, at a signal-to-noise ratio of 3. A GCE modified by copper-doped titanium dioxide nanoneedles exhibts good stability and has promising characteristics for the detection of AA.

Keywords: ascorbic acid, copper-doped titanium dioxide nanoneedles, electrochemical detection, glassy carbon electrode.

Ascorbic acid (AA), also called vitamin C, belongs to inhibitory neurotransmitters and has an important effect on the functioning of the renal, central nervous, hormonal, and cardiovascular systems [1]. In addition, AA is a vital component of the human diet and is clinically used in the prevention of scurvy. It can also help to produce collagen, a protein for the maintenance and development of cartilage, joint linings, bones, teeth, gums, skin, and blood vessels [2]. Therefore, the detection and investigation of AA is very important for biomedical chemistry and neurochemistry.

Electrochemical detection of organic biological molecules is a quite simple process, it requires a simple apparatus, it is a low-cost and fast analysis [3, 4]. Different electrodes have been proposed for the electrochemical detection of AA by the cyclic voltammetry. Pandey et al. [5] reported on the functionalized ormosil modified electrodes developed for this purpose. The ormosil modified electrodes were fabricated by encapsulating potassium ferricyanide/potassium ferrocyanide with an ormosil film derived from the composition of 3-aminopropyltrimethoxysilane, 2-(3,4-epoxycyclo-hexyl)ethyl trimethoxysilane and phenyltrimethoxy silane with Nafion/crown ether. The detection limit was 0.5 μM without any interference effect. Florou et al. [6] reported the electrochemical detection of AA based on a glassy carbon electrode (GCE) modified with a cellulose acetate polymeric film bearing 2,6-dichlorophenolindophenol (CA/DCPI-CME). The linear range was 0.02–1 mM and 0.1–6 mM AA for the CA/DCPI sensor hydrolyzed in KOH and $ZnCl_2$ solutions, respectively. A molecularly imprinted polypyrrole-modified pencil

graphite electrode [7] and an α-MnO_2 modified carbon black microelectrode [8] have also been developed for the electrochemical detection of AA. The linear range and detection limits were 0.25–7.0 mM, 0.074 mM and 0.001–4 mM, 0.6 μM, respectively, at a signal-to-noise ratio of 3. A $Cu_4(OH)_6SO_4$ nanorods modified biosensor exhibited good electrochemical properties for the AA determination with the linear range of 0.017–6 mM and the detection limit of 6.4 μM, [9]. Keeley et al. [10] reported the electrochemical determination of AA at the graphene nanosheets (GNSs) immobilised on the pyrolysed photoresist film (PPF) electrode. That electrode showed a linear range of 0.4–6 mM with a 0.12 mM detection limit. The electrochemical sensor for simultaneous detection of AA and other biological molecules was also reported using different electrodes, such as an activated roughened GCE [1], a mesoporous silica [11] and an L-cysteine self-assembled gold electrodes [12]. However, the detection of AA is often suffering from a low detection limit, selectivity, and low sensitivity. Therefore, it is essential to develop a reliable and efficient electrode with enhanced characteristics for the effective detection of AA.

At a modified GCE, Cu ions can be continuously recovered by the electrochemical oxidation or reduction processes, which mediates the final chemical oxidation or reduction of the target materials. In our previous research, a GCE modified by Cu germanate nanowires exhibited good electro-chemical performance for the AA detection in neutral solutions [13]. The linear range was 0.01–5 mM and the detection limit was 8.6 μM, at a signal-to-noise ratio of 3. In addition, Cu vanadate

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nanobelts have been synthesized by us by a simple hydrothermal process and used as the GCE modified materials for the electrochemical determination of AA [14]. The linear range and the detection limit were 0.001–2 mM and 0.14 μM and 0.38 μM for cyclic voltammogram peaks (cvp 1 and cvp 2), respectively. The results reveal that nanomaterials with Cu ions show great application potential for the electrochemical detection of organic molecules. It is these reasons that stimulated our interest for the synthesis and electrochemical detection of orgainc molecules using nanomaterials with Cu ions. In the present paper, a GCE modified with the Cu-doped TiO_2 nanoneedles was used for the electrochemical detection of AA. It is worth noting that copper-doped titanium dioxide nanoneedles have an intrinsic electrocatalytic activity. The detection limit, linear range, and correlation coefficient for the AA detection have been established.

MATERIALS AND METHODS

Copper-doped titanium dioxide nanoneedles were obtained via a simple hydrothermal process. In a typical synthesis procedure, Cu acetate, titanium butoxide ($C_{16}H_{36}O_4Ti$, AR grade), and sodium dodecyl sulfate, with a definite mass percentage, were dissolved in 60 mL of deionized water, under vigorous stirring. The mass ratio of Cu and titanium dioxide was 5:95. Then, the mixture was placed in a 100 mL autoclave with a Teflon liner. The autoclave was kept at 180°C for 24 h. Then the autoclave was cooled naturally. The light grey precipitates were filtered, washed several times with deionized water and dried at 60°C. Finally, light grey powders were obtained.

Suspension of copper-doped titanium dioxide nanoneedles was prepared by dispersing 10 mg of copper-doped titanium dioxide nanoneedles in 10 mL of dimethylformamide solvent. Prior to being modified by copper-doped titanium dioxide nanoneedles, a GCE with a diameter of 3 mm was polished to a mirror-like surface using polish paper with alumina pastes of 0.5 μm, and then cleaned thoroughly in an ultrasonic cleaner with alcohol and water, respectively. A GCE modified by copper-doped titanium dioxide nanoneedles was prepared by dipping 10 μL of copper-doped titanium suspension onto the surface of the GCE and dried at room temperature.

The morphology of the surface of the thus-modified GCE was analyzed using JEOL JSM-6490LV scanning electron microscopy (SEM). Electrochemical cyclic voltammograms (CVs) of AA were measured using CHI6046D electro-chemical working station in the potential range of -1.0 to 1.0 V, with the scan rate of 50–200 mVs^{-1} in 0.1M KCl and AA with different concentrations.

The thus-modified GCE, a saturated calomel electrode (SCE), and a platinum plate served as the working electrode, reference electrode, and counter electrode, respectively. All potentials were reported with respect to the SCE.

RESULTS AND DISCUSSION

The morphology of the surface of the thus-modified GCE is shown in Figure 1. A dense film composed of copper-doped titanium dioxide nanoneedles can be formed on the surface of the GCE. The length of a nanoneedle is about 10 μm. Its diameter vividly decreases from about 250 nm at the initating terminal to 20 nm at the end. Their surface is smooth. Therefore, the substrate of the GCE has no role in the electrochemical responses of AA at the GCE modified by copper-doped titanium dioxide nanoneedles.
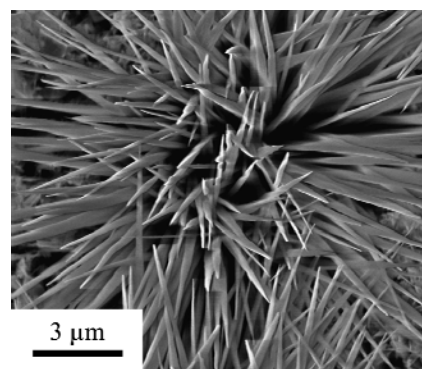

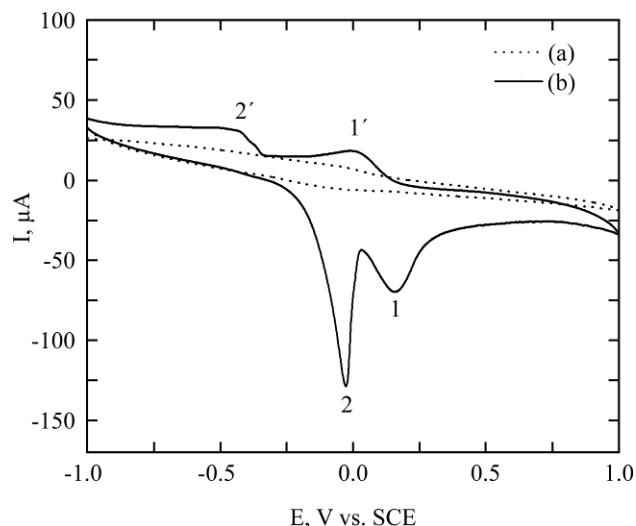**Fig. 1.** SEM image of GCE modified by copper-doped titanium dioxide nanoneedles.

Fig. 2. CVs of nanoneedles-modified GCE in different solution with scan rate 50 mVs^{-1}. (a) 0.1M KCl solution without AA; (b) mixed solution of 0.1M KCl and 2 mM AA.

The electrochemical activities of AA at the modified GCE are analyzed in KCl solution using the scan rate of 50 mVs^{-1}. The CVs of the GCE modified by copper-doped titanium dioxide nanoneedles in KCl solution of 0.1M with and without 2 mM of AA are shown in Figure 2. No CV peaks are observed from the CV at the nanoneedles-

modified GCE without AA (Fig. 2a) suggesting that the copper-doped titanium dioxide nanoneedles have no electrochemical activities in KCl solution of 0.1M without AA. The electrochemical activities at the GCE modified by copper-doped titanium dioxide nanoneedles in KCl solution without AA are similar to those at GCEs modified by Cu germanate nanowires and Cu vanadate nanobelts [13, 14]. Unlike the CV at the modified GCE, recorded in the absence of AA, two pairs of quasi-reversible CV peaks are observed from the CV curve at the nanoneedles-modified GCE in 0.1M KCl and 2 mM AA solutions (Figure 2b). Two anodic electrochemical CV peaks: cvp 1 and cvp 2, are located at +0.16 V and -0.03 V, respectively. Two cathodic electrochemical CV peaks: cvp 1′ and cvp 2′, are located at +0.01 V and -0.44 V, respectively. Generally speaking, an irreversible CV peak, or a pair of quasi-reversible CV peaks, is observed from the electrochemical responses of AA at different electrodes. For example, only an irreversible oxidation CV peak at +0.16 V was observed from the CV of AA at an activated roughened GCE in a buffer solution with the pH of 1.98 [1]. Zhang et al. [8] reported an irreversible oxidation CV peak located at 0.0 V at the α-MnO$_2$ modified carbon black microelectrode in AA solution. The irreversible oxidation CV peak was located at -0.017 V in a *buffer* (*p*hosphate-buffered saline) solution of 0.1M toward the oxidation of 0.5 mM AA at the GCE modified by Cu$_4$(OH)$_6$SO$_4$ nanorods [9]. However, no reproducible electrode response was obtained because of the fouling of the electrode surface by the adsorption of the oxidized product of AA. An irreversible oxidation CV peaks located at +0.33 V and +0.11 V were also observed at the bare Au electrode and L-cysteine Au electrode, respectively [12]. A pair of quasi-reversible CV peaks with the anodic peak at -0.55 V were observed from the CV of 0.5 mM AA in ionic liquid [15]. Different from the above reports, two pairs of electrochemical CV peaks were observed from the CV of AA at the modified GCE. The electrochemical CV peaks can only be observed from the mixed solution with KCl and AA at the GCE modified by copper-doped titanium dioxide nanoneedles showing that the CV peaks originate from AA.

AA can be reduced to form dehydroascorbic acid which was reported by Florou et al. [6]. Others reported that AA can be reduced on a platinum electrode in [bmim][BF$_4$] ionic liquid [15]. Hager et al. [16] analyzed the adsorption and desorption behavior of cysteine showing the adsorption and desorption processes of cysteine and cystine at the surface of a gold electrode at -0.45 V and -0.65 V, respectively. In our earlier research, two pairs of CV peaks were observed from the CV of AA in KCl solution at the GCE modified by Cu germanate nanowires [13]. Two anodic CV peaks (cvp 1 and cvp 2) were located at 0.202 V and 0.017 V, and two cathodic peaks (cvp 1′ and cvp 2′) were located at 0.068 V and -0.406 V, respectively. Similar to the electrochemical responses of AA at the GCE modified by Cu germanate nanowires – cvp 1 and cvp 1′ contribute to the oxidation-reduction process between AA and dehydroascorbic acid. Cvp 2 and cvp 2′ can be assigned to the adsorption-desorption process of AA and dehydroascorbic acid.

The electrochemical behavior of biological molecules at modified electrodes can be greatly affected by adjusting the scan rate. Figure 3 shows the CVs of 2 mM AA in 0.1M KCl solution at the GCE modified by copper-doped titanium dioxide nanoneedles using the scan rate in the range of 25 to 200 mVs^{-1}. With increasing the scan rate from 25 to 200 mVs^{-1}, the intensities of the electrochemical CV peaks also increase. The relation curve between the intensities of the CV peaks and scan rate is shown in the bottom-left part of Figure 3. There is a linear relationship between the intensities of the CV peaks and the scan rate. The correlation coefficients are 0.997 and 0.995 for cvp 1 and cvp 2, respectively. The results suggest that the electrochemical process between AA and dehydroascorbic acid can be controlled by adsorption [14, 17].

The electrochemical detection parameters including a correlation coefficient, a linear range and a detection limit have been analyzed in KCl solution with AA of different concentrations. Figure 4 shows the CVs of AA with the concentration range from 0.0005 to 2 mM at the nanoneedles-modified GCE. The relationship between the intensities of the CV peaks and AA concentration is shown in the bottom-left part of Figure 4. With the increase of the AA concentration, the intensities of the CVs of AA at the nanoneedles-modified GCE increase greatly. The regression equation, detection limit, correlation coefficient, and linear range are listed in Table 1. The linear range is 0.0005–2 mM. The correlation coefficients are 0.994 and 0.997 for cvp 1 and cvp 2, respectively. Solutions with low concentrations must naturally have an increasingly higher error. The correlation coefficients are 0.929 and 0.945 for cvp 1 and cvp 2, respectively, in the AA concentration range of 0.0005–0.1 mM. The detection limits are 0.37 μM and 0.25 μM for cvp 1 and cvp 2, respectively, at a signal-to-noise ratio of 3. Comparing with the electrochemical detection for AA at other electrodes [8–15], the GCE used in the present research shows a lower detection limit and a quite broad linear range.

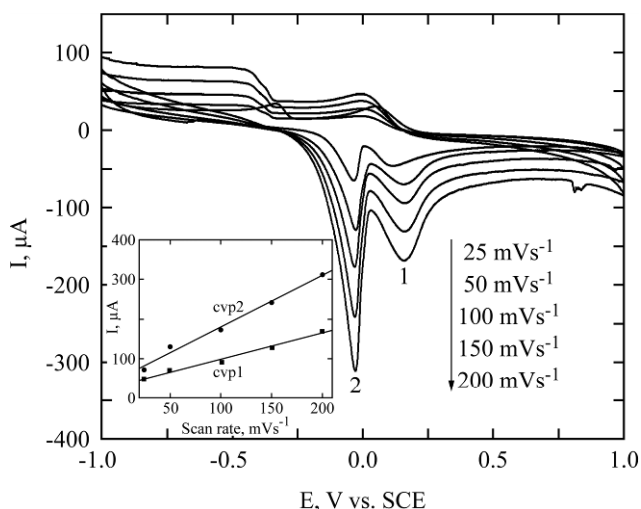The stability of the nanoneedles-modified GCE has been analyzed by the repeated measurements

Fig. 3. CVs of nanoneedles-modified GCE in mixed solution of 0.1M KCl and 2 mM AA using different scan rates. The inset in the bottom-left part is the relation between the scan rate and intensities of CV peaks.
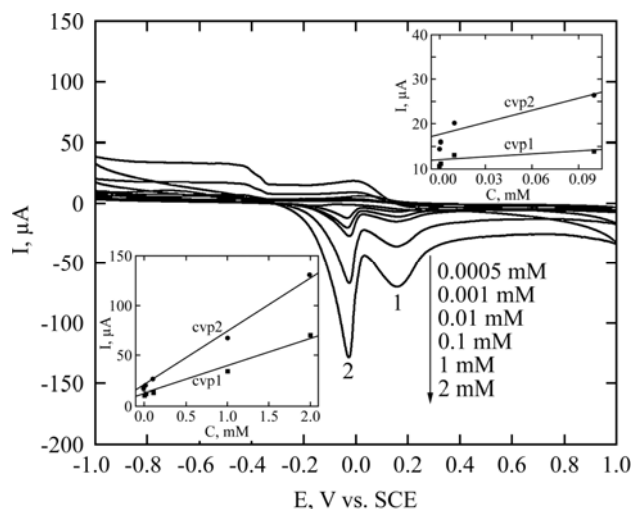
Fig. 4. CVs of AA with different concentrations at the nanoneedles-modified GCE: KCl, 0.1M, scan rate, 50 mVs^{-1}. The inset in the bottom-left part is the relation between the intensities of the CV peaks and AA concentration in the concentration range of 0.0005–2 mM. The inset in the upper-right part is the relation between the intensities of the CV peaks and AA concentration in the concentration range of 0.0005–0.1 mM.

Table 1. Analytical data of AA at the nanoneedles-modified GCE

CV peaks	Regression equation[a]	Correlation coefficient (R)	Linear range (mM)	Detection limit (μM)[b]
cvp 1	$I_p = 11.263 + 28.737C$	0.994	0.0005–2	0.37
cvp 2	$I_p = 20.895 + 54.053C$	0.997	0.0005–2	0.25

[a] Where I_p and C represent the peak current (μA) and AA concentration (mM).
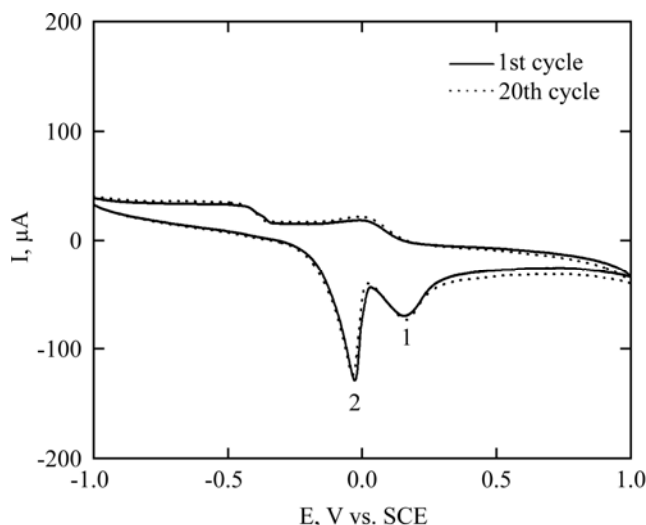[b] The detection limit of AA was analyzed using a signal-to-noise ratio of 3 (S/N=3).

Fig. 5. CVs of nanoneedles-modified GCE in mixed solution of 0.1M KCl and 2 mM AA recycling for the 1st and 20th time, respectively, with scan rate 50 mVs^{-1}.

using the same modified electrode. Figure 5 shows the CVs of 2 mM AA at the nanoneedles-modified GCE for the repeated measurements of the 1st and 20th time, respectively. The relative standard deviations are 4.29% and 2.39% for cvp 1 and cvp 2, respectively. The nanoneedles-modified GCE can be firmly adsorbed to the surface of the GCE. The nanoneedles-modified GCE can be used at least two weeks with only a slight decline of the electrochemical signal, hence demonstrating good stability.

In practice, a GCE modified by copper-doped titanium dioxide nanoneedles has been used for the determination of AA in tap water samples. The AA concentration in tap water was 5, 20 and 40 μM, respectively. The real sample measurements were performed at room temperature. The measured values were obtained from five separate measurements. The recoveries of AA were determined by standard addition. The results are listed in Table 2 suggesting that the nanoneedles-modified GCE is reliable and sensitive for the determination of AA.

Table 2. Electrochemical determination of AA using nanoneedles-modified GCE in tap water

Sample (Tap water)	Amount added (μM)	Amount found (μM) (average of five times)	Recovery (%)
1	5	4.85 ± 0.12	97.5
2	20	19.82 ± 0.26	99.2
3	40	41.21 ± 0.31	105.1

CONCLUSIONS

To sum up, a GCE modified by copper-doped titanium dioxide nanoneedles has been used for the electrochemical detection of AA in KCl solution. There are two pairs of quasi-reversible electrochemical CV peaks in the CV of AA in KCl solution at the nanoneedles-modified GCE. The anodic CV peaks (cvp 1 and cvp 2) are located at +0.16 V and -0.03 V, respectively. The cathodic CV peaks (cvp 1′ and cvp 2′) are located at +0.01 V and -0.44 V, respectively. The linear range is 0.0005–2 mM. The detection limits are 0.37 μM and 0.25 μM for cvp 1 and cvp 2, respectively. Comparing with the electrochemical detection for AA at other electrodes, the GCE used in the present investigation shows a lower detection limit and a broad linear range.

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Реферат

Был изготовлен стекловидный углеродный электрод (СУЭ), модифицированный наноиглами из диоксида титана легированного медью и использован для электрохимического определения аскорбиновой кислоты (АК) в растворе KCl. Две пары пиков тока на квазиобратимой электрохимической циклической вольтамперограмме пиков (ЦВП) расположены в +0,16 В, -0,03 В (ЦВП 1 и ЦВП 2) и +0,01 В, -0,44 В (ЦВП 1' и ЦВП 2'), соответственно. Связь между пиковым током и концентрацией АК линейна в диапазоне концентраций от 0,0005 до 2 мМ. Существует также линейная зависимость между пиком тока и скорости сканирования. Предел обнаружения составляет 0,37 мкМ и 0,25 мкМ для ЦВП 1 и ЦВП 2, соответственно, при соотношении сигнал-шум 3. СУЭ, модифицированный наноиглами из диоксида титана легированного медью, показывает хорошую стабильность и имеет многообещающие характеристики для применения при определении содержания АК.

Ключевые слова: аскорбиновая кислота, иглы диоксида титана легированные медью, электро-химическое определение, стекловидный углеродный электрод.